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Short communication

# Pirmenol inhibits muscarinic acetylcholine receptor-operated $K^+$ current in the guinea pig heart

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**Abstract**

We examined the effects of pirmenol and disopyramide on the muscarinic acetylcholine receptor-operated $K^+$ current ($I_{K.ACh}$) in atrial cells and on experimental atrial fibrillation in isolated guinea-pig hearts. In isolated atrial myocytes, both pirmenol and disopyramide concentration-dependently inhibited the $I_{K.ACh}$ induced by carbachol or intracellular loading of GTPγS. Their inhibitory effects on the carbachol-induced current were more potent than those on GTPγS-induced current, suggesting that these drugs inhibit $I_{K.ACh}$ mainly by blocking muscarinic receptors. In Langendorff-perfused hearts these drugs reversed the carbachol-induced decreases in effective refractory periods and atrial fibrillation threshold. These drugs may be useful for the prevention of vagally induced atrial fibrillation.


*Keywords:* $K^+$ current, muscarinic acetylcholine receptor-operated ($I_{K.ACh}$); Pirmenol; Atrial fibrillation; Disopyramide

## 1. Introduction

Atrial fibrillation is the commonest arrhythmia for which antiarrhythmic drugs are currently prescribed. The muscarinic acetylcholine receptor-operated $K^+$ current ($I_{K.ACh}$) plays an important role in the repolarization of the action potential as well as the maintenance of the resting potential in atrial cells (Kaibara et al., 1990). It has been reported that many antiarrhythmic agents, including class I, III and IV drugs, inhibit $I_{K.ACh}$ by different cellular mechanisms (Nakajima et al., 1989; Wu et al., 1994; Hara and Nakaya, 1995; Mori et al., 1995). The inhibitory effect on $I_{K.ACh}$ may be important for the treatment of vagally mediated atrial fibrillation (Ruffy, 1995).

Recently, pirmenol, a class I antiarrhythmic agent, has been shown to be clinically effective for paroxysmal atrial fibrillation (Atarashi et al., 1996). Since this drug has been shown to interact with atrial muscarinic receptors (Endou et al., 1992), it is expected to inhibit $I_{K.ACh}$. However, some antiarrhythmic drugs can inhibit $I_{K.ACh}$ more potently by depressing the function of the muscarinic $K^+$ channel itself and/or G proteins (Nakajima et al., 1989; Hara and Nakaya, 1995; Watanabe et al., 1996). Therefore, the first aim of the present study was to elucidate the cellular mechanism of $I_{K.ACh}$ inhibition by pirmenol in guinea-pig atrial cells, using patch clamp techniques. The second aim was to examine the effects of pirmenol on experimental atrial fibrillation in Langendorff-perfused guinea-pig hearts.

## 2. Material and methods

### 2.1. Patch clamp study

Single atrial cells were isolated from guinea-pig hearts by an enzymatic dissociation method, as previously described (Watanabe et al., 1996).

The tight seal, whole-cell voltage clamp technique was used for the recording of $I_{K.ACh}$ at $36.0 \pm 1.0$°C. The composition of the superfused HEPES–Tyrode's solution was (in mM): NaCl, 143; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; $NaH_2PO_4$, 0.33; glucose, 5.5 and HEPES–NaOH buffer (pH 7.4), 5.0. The pipette solution contained (in mM): potassium aspartate, 110; KCl, 20; $MgCl_2$, 1.0; $K_2$ ATP, 5.0; $K_2$ phosphocreatine, 5.0; EGTA, 10 and HEPES–KOH buffer (pH 7.4), 5.0 (pCa 8). GTP (100 μM) or GTPγS (100 μM) was also added to the pipette solution. The electrode (2–3 MΩ) was connected to a

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patch-clamp amplifier (CEZ-2300: Nihon Kohden, Tokyo) controlled by PCLAMP software (Axon Instruments, Foster City, CA, USA) on an IBM-compatible computer (Compaq Prolinea 4/50, Houston, TX, USA).

Current-clamp experiments were also performed in the whole-cell recording mode at $36.0 \pm 1.0$°C. The cells were stimulated by passing 2 ms currents through the pipette at the rate of 0.2 Hz.

### 2.2. Isolated heart study

The effects of pirmenol and disopyramide on the atrial effective refractory period and the atrial fibrillation threshold were examined in Langendorff-perfused isolated hearts, as previously described (Watanabe et al., 1996). The guinea-pig heart was perfused at constant pressure with the normal Tyrode's solution gassed with 95% $O_2$ and 5% $CO_2$ ($36.0 \pm 1.0$°C). The composition of normal Tyrode's solution was (in mM): NaCl, 125; KCl, 4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; $NaH_2PO_4$, 1.8; glucose, 5.5 and $NaHCO_3$, 25 (pH 7.4). The right atrium was stimulated with an external bipolar silver electrode using rectangular pulses of 2 ms duration at twice the diastolic threshold. The left atrial electrograms were recorded on a chart recorder with an additional bipolar electrode.

The atrial effective refractory period was determined by the standard extrastimulus technique. After every eighth basic right atrial stimulus ($S_1S_1$ 200 ms), an extrastimulus ($S_2$) was delivered with a shortening of the coupling interval ($S_1S_2$) in 5 ms steps until the $S_2$ produced no atrial activity. The effective refractory period was defined as the longest $S_1S_2$ that failed to elicit atrial activity in response to $S_2$.

The atrial fibrillation threshold was measured by rapid atrial electrical stimulation. The fibrillating current consisted of a train of 50 square wave pulses, 2 ms in duration at a frequency of 50 Hz for a duration of 1 s. The pulse train was delivered to the right atrium after every eighth basic paced beat. The atrial fibrillation threshold was defined as the minimum amount of current required to induce atrial fibrillation which was sustained for at least 15 s. The stimulator used in this study was unable to deliver a current greater than 2.0 mA. If atrial fibrillation could not be induced by the current less than 2.0 mA, the atrial fibrillation threshold was considered to be more than 2.0 mA.

### 2.3. Drugs and statistics

The drugs used in this study were carbachol chloride (Tokyo Kasei, Tokyo, Japan), pirmenol hydrochloride (Waner-Lambert, Tokyo, Japan) and disopyramide phosphate (Chugai, Tokyo, Japan). All values are presented as means ± S.E. Student's *t*-test was used for statistical analysis of the data. *P*-values of less than 0.05 were considered significant. $IC_{50}$ values were obtained using a Delta Graph Professional (Delta Point, Polaroid Computing, Tokyo, Japan).

## 3. Results

### 3.1. Effects of pirmenol and disopyramide on $I_{K.ACh}$ and action potential in atrial cells

After application of 1 μM carbachol to the bath solution, an outward $K^+$ current was rapidly activated at a holding potential of −50 mV (Fig. 1). Pirmenol and disopyramide inhibited the carbachol-induced $I_{K.ACh}$ in a concentration-dependent manner. The $IC_{50}$ values of pirmenol and disopyramide for inhibition of carbachol-induced $I_{K.ACh}$ were 0.17 and 0.98 μM, respectively. In GTPγS (100 μM)-loaded cells, antagonist-resistant, persistent outward currents were activated gradually even in the absence of any agonists (Fig. 1). Pirmenol and disopyramide also inhibited the GTPγS-induced current although the concentrations of these drugs needed to inhibit the GTPγS-induced current were much higher than those to inhibit the carbachol-induced $I_{K.ACh}$. The $IC_{50}$ values of pirmenol and disopyramide for inhibition of the GTPγS-induced currents were 30 and 80 μM, respectively. The inhibitory effects of pirmenol and disopyramide on these $I_{K.ACh}$ were almost completely reversible and the outward current reappeared upon washout of pirmenol or disopyramide.

The effects of pirmenol and disopyramide on the action potential recorded in the current clamp mode were examined. In the absence of carbachol, neither 1 μM pirmenol nor 10 μM disopyramide produced a significant increase in action potential duration in atrial myocytes (data not shown). Carbachol (1 μM) markedly shortened action potential duration at the 90% repolarization level to $11.8 \pm 1.3$% of the control ($58.8 \pm 2.7$ ms, $n = 24$) with an insignificant increase in the resting membrane potential. Pirmenol and disopyramide effectively reversed the carbachol-induced action potential shortening, as shown in Fig. 1. Pirmenol (1 μM) and disopyramide (10 μM) reversed the shortening of action potential duration at 90% repolarization level to $73.2 \pm 7.0$% and $72.4 \pm 8.6$% of the control, respectively.

### 3.2. Effects of pirmenol and disopyramide on experimental atrial fibrillation in isolated hearts

Under control conditions, atrial fibrillation could not be induced by a train of stimuli at an intensity of 2.0 mA in any of the hearts. After the application of 1 μM carbachol the atrial effective refractory period was significantly shortened, from $39.4 \pm 3.6$ to $15.0 \pm 1.2$ ms (Fig. 2) and the atrial fibrillation threshold was decreased to $0.9 \pm 0.2$ mA ($n = 9$). Addition of 1 μM pirmenol or 10 μM disopyramide reversed the shortening in effective refrac-

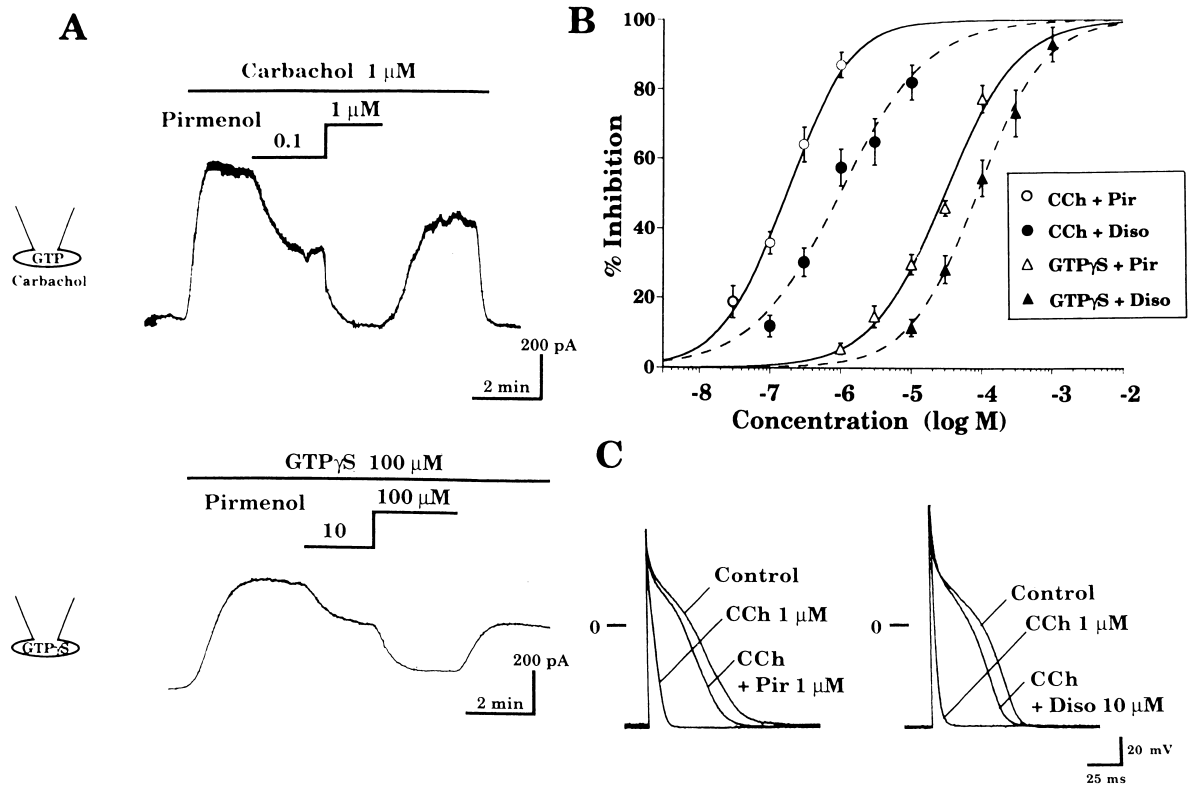


Fig. 1. Effects of pirmenol on the muscarinic acetylcholine receptor-operated $K^+$ current ($I_{K.ACh}$) and action potential in guinea pig atrial cells. Actual records showing the effects of pirmenol on the $I_{K.ACh}$ activated by extracellular application of 1 μM carbachol (CCh) or intracellular loading of 100 μM GTPγS are depicted in A. The cells were held at −50 mV. Extracellular application of drugs is shown by the lines above each original current trace. Concentration–response curves for the inhibitory effects of pirmenol and disopyramide on $I_{K.ACh}$ are shown in B. Percent inhibition of the outward current is indicated on the ordinate and the concentrations of pirmenol or disopyramide are on the abscissa. Values are expressed as means ± S.E. of 3 to 14 experiments. Superimposed records of action potentials obtained before (Control) and after exposure to 1 μM CCh and 1 μM CCh plus 1 μM pirmenol (Pir) or 10 μM disopyramide (Diso) are shown in C.

tory period (Fig. 2). After the administration of pirmenol or disopyramide, atrial fibrillation could no longer be induced in the carbachol-treated hearts, indicating that the atrial fibrillation threshold was increased to a level higher than 2.0 mA.

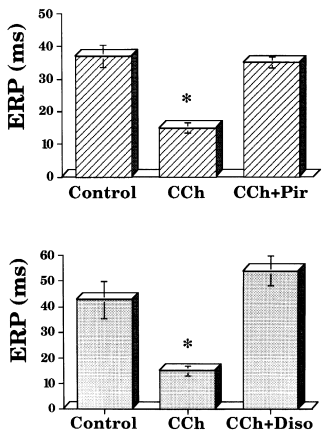


Fig. 2. Effects of pirmenol or disopyramide on the carbachol (CCh, 1 μM)-induced decreases in effective refractory period (ERP) in guinea-pig isolated hearts. The definition of ERP is given in the text. The values are expressed as means ± S.E. for 4 or 5 hearts. $^*P < 0.05$ versus control.

## 4. Discussion

Recent progress in the non-pharmacological therapy of cardiac arrhythmias with catheter ablative techniques or implantable cardioverter defibrillator has had a great impact on pharmacological therapy with antiarrhythmic drugs. However, paroxysmal atrial fibrillation is still an important target for the pharmacological therapy. Therefore, it would be of pharmacological significance to evaluate the effects of antiarrhythmic drugs on experimental atrial fibrillation.

It is well-known that vagal stimulation can easily elicit atrial fibrillation in vivo (Von Euler and Scanlon, 1987). The activation of $I_{K.ACh}$ can result in the shortening of action potential duration and effective refractory period, and enhance the susceptibility to atrial fibrillation. It is reported that many class I, class III and IV antiarrhythmic drugs inhibit $I_{K.ACh}$ in atrial cells (Nakajima et al., 1989; Wu et al., 1994; Hara and Nakaya, 1995; Mori et al., 1995; Watanabe et al., 1996). Two mechanisms by which these antiarrhythmic drugs inhibit $I_{K.ACh}$ have been proposed; some drugs block the muscarinic receptors and others inhibit the muscarinic $K^+$ channel itself and/or GTP-binding proteins. Sotalol belongs to the former group (Mori et al., 1995) whereas quinidine, cibenzoline, bepridil and

amiodarone belong to the latter group (Nakajima et al., 1989; Wu et al., 1994; Hara and Nakaya, 1995; Watanabe et al., 1996). Some class I and III drugs such as disopyramide, E-4031 and MS-551 inhibit $I_{K.ACh}$ mainly by blocking the muscarinic receptors (Nakajima et al., 1989; Wu et al., 1994; Mori et al., 1995). Consistent with previous studies, higher concentrations of disopyramide were needed to inhibit the GTPγS-induced $I_{K.ACh}$ than those to inhibit the carbachol-induced $I_{K.ACh}$.

Pirmenol is a class $I_a$ antiarrhythmic drug whose inhibitory action on the maximum upstroke velocity of the action potential is three times more potent than that of disopyramide in guinea-pig papillary muscles (Nakaya et al., 1988). Pirmenol was shown to inhibit not only the $Na^+$ channel but also the $K^+$ channel in rabbit Purkinje fibers (Reichardt et al., 1990). Pirmenol (0.5–3 μM) depressed the delayed rectifying current ($i_x$) in a concentration-dependent manner. As already mentioned, some antiarrhythmic drugs with $K^+$ channel blocking action can inhibit $I_{K.ACh}$ by depressing the function of the $K^+$ channel itself and/or G proteins. Therefore, we examined the effects of pirmenol on the carbachol- and the GTPγS-induced $I_{K.ACh}$ in isolated guinea-pig atrial cells. As observed with disopyramide, pirmenol more potently inhibited the carbachol-induced current than the GTPγS-induced one, indicating that pirmenol also inhibits $I_{K.ACh}$ mainly by blocking the muscarinic receptors. The inhibitory effect of pirmenol on the carbachol-induced $I_{K.ACh}$ was about 6 times more potent than that of disopyramide on the molar basis. The $IC_{50}$ value of pirmenol for inhibiting $I_{K.ACh}$ in atrial cells is far less than that for depressing $V_{max}$ by 20% in guinea-pig papillary muscles (approximately 10 μM) (Nakaya et al., 1988). Furthermore, the plasma concentration of pirmenol in the human at 2 h after a single oral dose of 200 mg is $1.40 \pm 0.52$ μg/ml (Atarashi et al., 1996). Since the plasma protein binding of this drug was reported to be about 85% (Chang, 1987), the free pirmenol concentration might correspond to 0.5 μM. Therefore, pirmenol is expected to potently inhibit $I_{K.ACh}$ in the clinical setting.

In the present study, administration of pirmenol or disopyramide prevented the atrial fibrillation induced by a combination of the muscarinic agonist and high-frequency atrial stimulation. We cannot exclude the possibility that the $Na^+$ channel blocking action of these drugs might also contribute to the antifibrillatory effect. However, the inhibitory effects of pirmenol and disopyramide on $I_{K.ACh}$ may play an important role in the establishment of the antifibrillatory effects, because the increase in the atrial fibrillation threshold was accompanied by reversal of refractory period shortening.

The anticholinergic activity of antiarrhythmic drugs may produce untoward as well as therapeutic effects. Antiarrhythmic drugs having anticholinergic activity may prevent the occurrence of vagally induced atrial fibrillation. However, the anticholinergic action may lead to facilitation of atrioventricular conduction and an increase in the ventricular rate during atrial fibrillation. Therefore, this effect could be dangerous in the clinical setting. In addition. anticholinergic activity can lead to extracardiac adverse effects such as dry mouth, constipation and urinary retention. However, it has been shown that pirmenol shows a several times lower affinity for glandular muscarinic $M_3$ receptors than for cardiac muscarinic $M_2$ receptors, suggesting that pirmenol may exert a smaller anticholinergic effect in peripheral tissues (Endou et al., 1992).

In conclusion, pirmenol may prevent parasympathetic-type paroxysmal atrial fibrillation by potently inhibiting $I_{K.ACh}$.

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